Symposium: Myosin Motors in Vitro and in Cells

3163-Symp
Unravelling the Properties of Single α-Helical Domains in Myosin and other Proteins
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In most proteins, α-helices are stabilised by interactions with neighbouring secondary structure elements (e.g. coiled-coils). However, we recently showed that many proteins contain single α-helical (SAH) domains, which are stable in isolation and commonly inserted between two different functional domains. SAH domains are rich in arginine, lysine and glutamic acid residues. Their stability arises from the many potential (i, i ± 4) and (i, i ± 3) intrahelical interactions between either R and E, or K and E.
To date, the best-studied SAH domains are those from myosins 6 and 10, and the Dictyostelium myosin myoM, where they are likely to form part of the functional lever. A SAH domain is also predicted for myosin 7a. We have shown that the SAH domain can functionally substitute for the canonical lever in myosin 5a in vitro. In cells, myosin 10 missing its SAH domain still moves to the tips of filopodia but with a reduced velocity.
We have now determined that the SAH domains unfold at forces of less than ~50 pN using single molecule force microscopy. Moreover, SAH domains unfold non-co-operatively during thermal melting, and have a high propensity to refold completely when the temperature is returned back to 10 °C, measured by circular dichroism. Molecular simulations agree with all the experimental data available, confirming the surprising stability of SAH domains and revealing unique properties that set the SAH domains apart. Among these is the ability to recover helical conformation rapidly after being extended through application of force.
Thus SAH domains appear to function as 'stretchable' elements, unfolding and rapidly refolding at relatively low forces. In myosins, this may enable these motors to hold on to and traffic their cargoes in the crowded environment of the cell.

3164-Symp
Myth-Ferm Myosins have Roles in Regulating Actin Polymerization
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The MyTH-FERM (MF) myosins are a subgroup of actin-based motors characterized by the presence of one or two adjacent MyTH (myosin tail homology 4)/FERM (band 4.1, ezrin, radixin, moesin) domains in their C-terminal tail regions. Although the MF myosins Myo7, Myo10, Myo15 and Myo44 are phylogenetically distinct, they have several characteristics in common. Each of these MF myosins has a striking localization to the tips of structures formed by parallel actin bundles such as filopodia and stereocilia and the different myosins play a role in the extension of either filopodia (Myo10) or stereocilia (Myo7A, Myo15) in Metazoa and filopod extension in Amoebozoa (Myo7). Their shared roles in the extension of parallel actin bundles is consistent with each of these myosins transporting or anchoring regulators of actin polymerization to the growing tip. Surprisingly, the amoeboid Myo44 is not needed for the formation of filopodia. However, Myo44 is rapidly recruited to the plasma membrane following chemotactic stimulation, consistent with its role in mediating the transmission of chemotactic signals that generate cell polarity to the actin cytoskeleton. Myo44 is necessary for the rapid activation of actin polymerization (~5 sec) following chemotactic stimulation and is required for activating Ras, a critical small GTPase that promotes the rapid, localized polymerization of actin at side of the cell closest to the stimulus. These results establish a new role for MF myosins in signaling to the actin cytoskeleton and establish a general role for this group of myosins in regulating actin polymerization.
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3165-Symp
Directed Actin Assembly and Contractility
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University of Grenoble, Grenoble, France.
The organization of actin filaments into higher-ordered structures governs eukaryotic cell shape and movement. Global actin network size and architecture is maintained in a dynamic steady-state through regulated assembly and disassembly. We have developed a micropatterning method that enables the spatial control of actin nucleation sites for in vitro assays (Reymann, Nat Mat, 2010). These actin templates were used to evaluate the response of oriented actin structures to myosin-induced contractility. We determine that myosins selectively contract and disassemble anti-parallel actin structures while parallel actin bundles remain unaffected. In addition, the local distribution of nucleation sites and the resulting orientation of actin filaments regulate the scalability of the contraction process. This ''orientation selection'' mechanism for selective contraction and disassembly reveals how the dynamics of the cellular actin cytoskeleton is spatially controlled by actomyosin contractility. Further application of the micropatterning method will be presented in particular recent data on the reconstitution of a lamellipodium-type of actin organization and the fabrication of three-dimensional electrical connections by means of directed actin self-organization.

Symposium: Biophysics of Genetic Switches

3166-Symp
A Quantitative Narrative for the Life Cycle of Bacteriophage Lambda
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The goal of my lab is to form a quantitative narrative for the fundamental processes driving the living cell. This narrative is built upon precise measurements performed in individual cells, at the level of individual molecules and discrete events in space and time. To achieve this level of detail, we are using a synthesis of approaches: classical molecular biology and biochemistry; single-cell and single-molecule fluorescence microscopy; advanced image- and data analysis algorithms. By using simple, coarse-grained theoretical models, we are able to distill our result into general principles, which can then be compared across organisms. I will present a few examples from our work, focusing on the study of the life cycle of bacteriophage lambda, a simple paradigm for cellular decision making.

3167-Symp
Long-Range DNA Looping in the Lambda Genetic Switch
Keith Shearwin, Lun Cui, Iain Murchland, Ian B. Dodd.
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In eukaryotes, transcription is often controlled by enhancers, DNA sequences that can be located many kilobases away from the promoter. These long-range effects involve DNA looping and direct contact between the enhancer and the promoter. However, the complexity of eukaryotic transcriptional control has precluded a detailed molecular understanding of enhancer-promoter complexes, so that it is not known how their formation can be efficient and specific. Long-range interactions are also critical in regulation of transcription by the CI repressor of the E. coli bacteriophage λ. A CI tetramer bound to the λ OR 'switch' region and a tetramer bound at the distal OL site interact cooperatively to form a CI octamer and a 2.3 kb DNA loop. We have shown that CI looping also can activate transcription by allowing the C-terminal domain of the α subunit of the RNA polymerase bound at OR to contact a DNA site adjacent to the distal CI sites at OL. We have developed a physicochemical model of our in vivo data and use it to show that the observed activation is consistent with a simple recruitment mechanism, where the α-C-terminal domain to DNA contact need only provide a modest amount of additional binding energy for RNA polymerase. Structural modelling of this complete enhancer-promoter complex reveals how the contact is achieved and regulated, and suggests that distal enhancer elements, once appropriately positioned at the promoter, can function in essentially the same way as proximal promoter elements.

3168-Symp
Genetic Switches and Transcriptional Regulation: Insights from Single Molecules
Laura Finzi.
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The lambda repressor-mediated DNA loop governs the epigenetic switch from lysogeny (quiescence) to lysis (virulence). Using magnetic tweezers, tethered particle and atomic force microscopy, we characterized how specific and non-specific binding as well as negative supercoiling allow the loop-based lambda switch to operate across a wide range of repressor concentrations and still switch efficiently to lysis. A similar interplay of specific and non-specific binding operates in the regulatory function of the disc-shaped 186 bacteriophage repressor heptamers which, by looping and wrapping DNA, have many mechanisms found in different transcriptional factors from bacteria to eukaryotes.